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Comparison of Bioassay and Enzyme-Linked Immunosorbent Assay for Quantification of *Spodoptera frugiperda* Nuclear Polyhedrosis Virus in Soil[1]

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Standard curves with known amounts of *Spodoptera frugiperda* nuclear polyhedrosis virus (NPV) in soil were established with a bioassay and with an enzyme-linked immunosorbent assay (ELISA). The bioassay detected as few as 4×10^4 polyhedral inclusion bodies (PIB)/g sandy soil and <10 PIB/g soils with large amounts of silt or clay. The ELISA detected as few as 360 PIB/g in all three soil types, and absorbance values were inversely related to the amount of clay. Results of the bioassay and ELISA were significantly ($P < 0.01$) correlated for natural NPV from field samples of silt ($R = 0.961$) and sandy soil ($R = 0.723$). Soil samples from Louisiana pastures and corn fields contain up to 7.6×10^4 PIB/g, and 2×10^4 PIB/g are commonly present. © 1985 Academic Press, Inc.

KEY WORDS: *Spodoptera frugiperda; Spodoptera frugiperda* Nuclear Polyhedrosis Virus; bioassay; enzyme-linked immunosorbent assay; soil, virus in.

INTRODUCTION

Quantification of nuclear polyhedrosis viruses (NPV) in soils is of primary importance to epizootiology of these microorganisms because soil is their most important abiotic reservoir (Jaques, 1977). For example, the amount of NPV in soil before seasonal *Spodoptera frugiperda* infestations in pastures is the most important factor that determines prevalence of the disease (Fuxa and Geaghan, 1983). Due to the importance of this reservoir, several methods have been used to detect NPV in soil, including bioassay (Jaques, 1967), light microscopy (Hukuhara and Namura, 1971; Evans et al., 1980), and scanning electron microscopy (Hukuhara, 1972).

Enzyme-linked immunosorbent assay (ELISA) is a technique that could have advantages over those previous methods if it can be used for quantification of NPV in soil. ELISA is likely to be more sensitive and specific than microscopy, and quicker and cheaper than bioassay. Several researchers have successfully detected NPV in laboratory-reared host insects (Kelly et al., 1978; Crook and Payne, 1980; Langridge et al., 1981; Volkman and Falcon, 1982; McCarthy and Henchal, 1983) and in field-collected insects (Longworth and Carey, 1980). However, ELISA apparently has not yet been used for detection of virus in a host insect's habitat.

One of the major questions about ELISA is whether its results will accurately reflect

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functional or biological activity (Rabin, 1980). This question is relevant to NPVs in insects, as well as to other systems for which ELISA is potentially useful, because detection of polyhedrin or virion components does not necessarily mean that the virus is viable and infective.

The purpose of the present research was to determine (1) whether the ELISA is sensitive enough to detect NPV of *S. frugiperda* in Louisiana soils, and (2) how well quantification of natural soil NPV by ELISA correlates with that by bioassay.

MATERIALS AND METHODS

Soil sampling. Soil samples were collected in 1982 from pastures or corn fields at three locations in southeastern Louisiana: Idlewild Experiment Station near Clinton, St. Gabriel Experiment Station, and 6 km east of Hammond. All the pastures and fields had a history of frequent infestations by *S. frugiperda*. Epizootics of NPV in *S. frugiperda* occurred in many of the pastures and fields during 1979–1982, but there was no NPV epizootic in any other insect species during that time. Soil compositions at the three sites (Table 1) were determined at the Louisiana State University soil laboratory.

Four soil samples were collected before the *S. frugiperda* season from each pasture or field, as described previously (Fuxa and Geaghan, 1983). The core sampler was 7.5-cm deep. Samples were air-dried, ground to a fine consistency with mortar and pestle, and stored at −6°C.

Soil bioassay. Soil samples were bioassayed with a leaf-disc method with 1-day-old *S. frugiperda* larvae, as described previously (Fuxa and Geaghan, 1983). A subsample of the soil was homogenized in distilled water, centrifuged, and resuspended in 0.05% Triton X-100. Test insects (60/sample) were placed on 5-mm browntop millet (*Panicum ramosum*) leaf discs which had been dipped in the suspension and dried, and they were transferred to artificial diet after 2 days. Controls were treated identically except that the left disc was dipped only in 0.05% Triton X-100. The insects were checked for mortality, and NPV disease was confirmed by observation of polyhedral inclusion bodies (PIB) by phase-contrast microscopy.

TABLE 1
SOIL COMPOSITION AT SAMPLE SITES IN LOUISIANA

Location	Percentage		
	Sand	Silt	Clay
Idlewild	15	71	14
Hammond	45	36	19
St. Gabriel	9	50	41

Standard curves were generated with known amounts of NPV added to the three soils which had been decontaminated by autoclaving. NPV was produced in laboratory-reared *S. frugiperda*, semipurified by filtration and tabletop centrifugation, and counted on a hemacytometer. For each dose 15 ml of NPV suspension was mixed with 55 g of the decontaminated soil. These soil samples were then air-dried and bioassayed as above. The bioassay standard curves had one replicate/dose.

ELISA procedures. The same field soil samples, but different subsamples, were used for ELISA as in the bioassays. In order to dissolve NPV polyhedrin, if present, 10 g of soil was suspended in 10 ml distilled water for 1 hr at 80°C. The suspension was cooled to room temperature in an ice-water bath, and 5 ml of Tween 20 was added along with 10 ml of alkaline saline (0.2 M Na_2CO_3, 0.3 M NaCl, *p*H 10.9) containing 0.01 M EDTA. The *p*H was adjusted to 12.0 with 3 N NaOH. The suspension was mixed thoroughly, incubated 2 hr (not overnight) at 4°C, and centrifuged 20 min at 2500 rpm in a tabletop centrifuge. The supernatant was filtered through No. 2 filter paper, and its *p*H was adjusted to 7.4 with 1 N HCl.

Monoclonal and rabbit anti-polyhedrin antibodies were used in the ELISA. The monoclonal antibody, prepared by the third

author in clone 1003, is specific for baculovirus polyhedrin but reacts with polyhedrin from many baculoviruses, including that from *S. frugiperda* NPV. The rabbit anti-polyhedrin antibody was kindly provided by Dr. Loy Volkman (Department of Entomology and Parasitology, University of California, Berkeley).

The ELISA procedure was modified from Volkman and Falcon (1982). The monoclonal antibody was diluted 1:1000 in 0.05 M carbonate buffer, *p*H 9.6. This solution (200 μl) was added to each well of a flat-bottom, Immulon-1 Microelisa plate (Dynatech Laboratories) with a Gillman Pipetman P1000 syringe and incubated. All incubations were overnight at 4°C or 3 hr at 37°C unless stated otherwise. After this and all subsequent incubations the wells were washed three times by aspirating them and adding 200 μl of the wash solution (0.15 M NaCl, 0.1% Tween 20) for at least 3 min per wash. Then 200 μl of the test sample (i.e., the soil supernatant, above) was added to each well and incubated. The wells were washed, and 200 μl of rabbit anti-polyhedrin, diluted 1:250 in PBS-T-PVP (0.01 M phosphate-buffered saline, *p*H 7.4, 0.05% Tween 20, 2% polyvinyl pyrrolidone-10, 0.2% ovalbumin), was added to each well and incubated. After another wash 200 μl of enzyme-coupled goat anti-rabbit IgG, diluted 1:250 in PBS-T-PVP, was added and incubated. The wells were washed again, and 200 μl of the substrate, *p*-nitrophenyl phosphate (1 mg/ml in 0.001 M $MgCl_2$, 0.001 M $ZnCl_2$, 0.1 M glycine buffer, *p*H 10.4) plus 0.01 M EDTA were added to each well and incubated 1 hr at 30°C. (Note: EDTA in this step reduced the sensitivity of the ELISA and should not be used in future research. However, its inclusion here did not affect relative results or conclusions drawn from the study.) Then 50 μl of 3 N NaOH was added to each well to stop the reaction and stabilize the color. Care was taken to stop the reaction after exactly 1 hr in each well. The optical density of the reaction product in each well was measured with a Bio-Tek EIA Reader Model EL307 at 405 nm.

Standard curves were determined by ELISA for the three soil types in Table 1. NPV was produced as above, and for each dose 6 ml of NPV suspension was mixed with 50 g decontaminated soil. These soil samples were air-dried and assayed with the ELISA procedure. For each curve there was one soil sample per dose, and that sample was assayed with six replicates (three runs of the ELISA with two wells/run).

Replication and controls for field soil samples. Each field-collected soil sample was subjected to one to four bioassays with a negative control and a positive standard soil (i.e., a known amount of NPV present) included with each run of the assay. For the ELISA there were six wells/soil sample, and controls between runs (plates) included a negative (decontaminated) control soil, a positive standard soil, a relatively low positive natural soil from the previous run, and a relatively high such soil (three wells for each of the four controls).

RESULTS

The bioassay standard curves indicated that as few as 4×10^4 PIB/g were detected in sandy soil (Hammond), and fewer than 10 PIB/g were detected in soils with predominantly silt or clay (Idlewild and St. Gabriel, respectively) (Fig. 1). Regression lines were fit to all the transformed data points for Idlewild and St. Gabriel and to the nonzero data points for Hammond. The regression equations were $y = 0.433x + 2.46$ (Idlewild), $y = 0.167x + 3.07$ (St. Gabriel), and $y = 0.167x - 10.40$ (Hammond).

The ELISA standard curves indicated that as few as 360 PIB/g were detected for all three soil types, although absorbance values were consistently inversely related to clay content of the soil (Fig. 2, Table 1). Regression lines were fit to the data in Figure 2 up to the point where the lines curved sharply upward, at which point the curves were hand-drawn. The regression

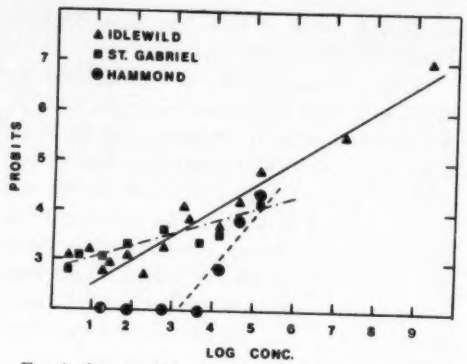

FIG. 1. Standard bioassay curves of known amounts of *Spodoptera frugiperda* NPV in decontaminated soil for three soil types (locations). The x axis represents the log of PIB per gram of soil, and the y axis represents percentage infection transformed to probits.

equations for the straight portion of each line were $y = -0.002x + 0.48$ (Idlewild), $y = 0.030x + 0.22$ (Hammond), and $y = -0.018x + 0.22$ (St. Gabriel). Straight regression lines did not adequately fit all data points for any of the three curves even when either or both variables were subjected to log transformation. All three lines curved upward sharply at ca. 3.6×10^8 PIB/g soil. Results of preliminary experiments indicated that all three lines would continue at ca. 0 slope down to lower doses tested (100 PIB/g for Idlewild and St. Gabriel soils, 7 PIB/g for Hammond). Absorbance was always 0 for negative control soils (decontaminated soil with no NPV added).

The bioassay and ELISA results were significantly correlated for field samples of soil NPV from Idlewild and Hammond (Fig. 3), but not from St. Gabriel. Correlation coefficients (R) were 0.513 ($n = 5$) for St. Gabriel soil (not significant at $P < 0.05$), 0.961 for Idlewild ($n = 7$), 0.723 for Hammond ($n = 21$), and 0.623 for all three combined ($n = 33$). The latter three R values are all significant at $P < 0.01$. The nonsignificant results with St. Gabriel soil were probably due to the low number of samples and low amounts of NPV in all the samples. The ELISA absorbance values for natural NPV in soil were all lower than those for the standard curves for all three soil types (Figs. 2, 3; the largest absorbance for a St. Gabriel field sample was 0.032). The regression equation for the combined Hammond–Idlewild data is $y = 0.011x + 0.003$, and that for all three data sets combined is $y = 0.010x + 0.002$. The regression equations for data in Figure 3 are $y = 0.028x - 0.069$ (Idlewild) and $y = 0.009x + 0.011$ (Hammond).

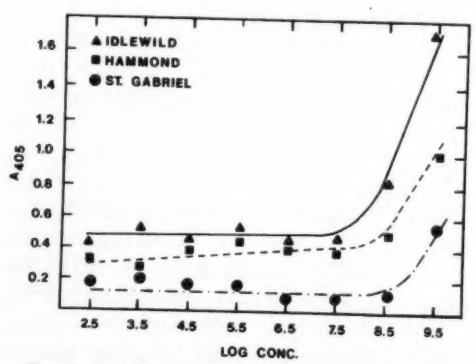

FIG. 2. Standard ELISA curves of known amounts of *Spodoptera frugiperda* NPV in decontaminated soil for three soil types (locations). The x axis represents the log of PIB per gram of soil, and the y axis is absorbance at 405 nm.

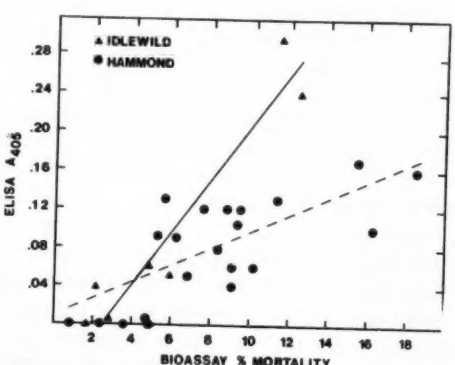

FIG. 3. Correlation of the results of ELISA vs bioassay for quantification of naturally occurring *Spodoptera frugiperda* NPV in field soil samples.

DISCUSSION

Results of the ELISA correlated well with biological activity (bioassay results) of natural *S. frugiperda* NPV in soil. It is likely that the virions in soil lose infectivity at approximately the same rate that polyhedrin deteriorates because the bioassay and the ELISA, results of which were significantly correlated, detect infectious virions and polyhedrin, respectively. The correlation also adds evidence that the *S. frugiperda* NPV was the only occluded baculovirus present in large quantities in the soil samples.

The ELISA detected NPV in soil but cannot be used directly to quantify NPV in soil with the present materials and methods. One reason is that the ELISA gave different results for NPV mixed with soil in the laboratory as opposed to that occurring naturally in field soil. ELISA produced absorbance values which were consistently larger for the standard curves than for natural soils, while the bioassay indicated that the amounts of NPV in field soil samples were within the range of ELISA standard curve doses or down to the range of doses in preliminary experiments. Another reason that the present ELISA cannot be used directly for quantification is that all three ELISA standard curves have a slope ca. equal to 0 in the range of natural amounts of NPV. Thus the ELISA was unable to distinguish different amounts of NPV below 3.6×10^8 PIB/g in soil with NPV added artificially (Fig. 2) even though it apparently distinguished different amounts in soil with naturally occurring NPV (Fig. 3). The reason for this difference is unknown.

It is possible that the ELISA could be used to indirectly quantify NPV in soil with the present materials and methods and thus be used in epizootiological studies. ELISA results can be converted to corresponding bioassay values with the regression equations in Figure 3, and bioassay values can, in turn, be converted to PIB per gram of soil (Fig. 1). Bioassay would provide a better, more direct quantification, but it is a more laborious procedure.

There are reasons to believe that the bioassay provides a reasonable estimate of the amount of *S. frugiperda* NPV in soil. Unlike the ELISA, bioassay results from natural soils can be related to the bioassay standard curves. Also, the transformed bioassay data fit straight regression lines just as they do in more straightforward dose–mortality research with pathogens. Finally, estimates of NPV in soil for other insects agree with out results. Jaques (1975) found 2.5×10^3–5×10^4 PIB/g *Trichoplusia ni* NPV in nontreated check plots early in an experiment with cabbage, and Thompson and Scott (1979) found an average of 5.4×10^3 PIB/cm^3 soil (and a maximum $>10^5$) for *Orgyia pseudotsugata* NPV in a forest. Based on the bioassay standard curves and field sampling during 1980–82 (Fuxa, unpubl.), there is an average of 2×10^4 PIB/g soil ($n = 38$) of *S. frugiperda* NPV in southeastern Louisiana pastures and fields with a history of infestations by the insect. The maximum observed was 7.6×10^4 PIB/g in a corn field and 7.5×10^4 PIB/g in a pasture.

The different soil types affected the ELISA differently from the bioassay (Figs. 1, 2). ELISA absorbances were inversely related to the percentage of clay in the soil, probably because microorganisms in general (Alexander, 1977), and NPV PIBs in particular (Evans et al., 1980), are thought to adhere more to clay particles than silt or sand. The ELISA procedure involved more purification for treatment of the soil than the bioassay did. Thus clay particles were removed before the ELISA procedure and probably carried some polyhedrin away from the supernatant and filtrate. On the other hand, some clay and perhaps silt particles are likely to have reached the leaf discs in the bioassay, carrying NPV with them.

Further research and refinements of the ELISA are necessary for the monitoring of

insect viruses in field soil. The present research indicates that the method is feasible for detection of small amounts of NPV polyhedrin in soil but cannot yet be used directly for quantification.

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